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Malaysian fermented shrimp paste (*belacan*): A source of potential probiotic lactic acid bacteria

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Abstract

In the present work, lactic acid bacteria (LAB) isolated from Malaysian fermented shrimp paste, locally known as *belacan*, were screened for their probiotic potential. Seventeen isolates were characterised after a preliminary subtractive screening based on morphology (catalase-negative and Gram-positive cocci/bacilli). The isolates were evaluated based on their tolerance towards the gastrointestinal environment, haemolytic properties, antagonism effect against selected pathogens, and antibiotic resistance patterns. The isolates were also molecularly identified *via* 16S rRNA sequencing. Out of 17, three isolates (BE3, BE7, and BE16) demonstrated tolerance to pH 2.5 (survival rates above 90%) and 0.3% bile salts (survival rates above 50%). Further screening performed on the three isolates indicated that all strains did not show undesirable haemolytic activities, and could inhibit the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhimurium to varying degrees. Additionally, the isolates were susceptible to ampicillin and chloramphenicol antibiotics, and resistant to nalidixic acid, streptomycin, and vancomycin antibiotics. The 16S rRNA gene sequence analysis identified the isolates as *Lactobacillus plantarum* with 98, 100, and 99% similarity for BE3, BE7, and BE16, respectively. Therefore, these findings suggested that LAB isolated from Malaysian fermented shrimp paste exhibited promising probiotic properties.

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Introduction

The word probiotic was introduced in 1953 by Werner Kollath, a German scientist, as "active substances that are essential for the healthy development of life" (Gasbarrini *et al.*, 2016). Although the word has been redefined several times, the recent most widely accepted definition across the globe is "live microorganisms which when administered in adequate amounts confer health benefit on the host" (FAO/WHO, 2002). Probiotics exert benefits, such as aiding in better health, maintaining the balanced ratio of normal flora in the intestine, and elevating the resistance of the host against pathogens invasion (Tripathi and Giri, 2014).

Many studies have discovered potential probiotics in food, and the Food and Agriculture Organisation and World Health Organisation (FAO/WHO, 2002) have set out a systematic approach to evaluate them. This includes the species' ability to resist the destructive level of gastric acidity and bile acid produced in the digestive tract. Moreover, the potential probiotics must be capable of producing antimicrobial activity against common pathogenic bacteria in the digestive system, besides being resistant to antibiotics. Consequently, their haemolytic properties must also be evaluated. The most investigated probiotics isolated from fermented foods are lactic acid bacteria (LAB), as they are the dominant microflora in fermented food besides being involved in fermentation processes (Abushelaibi *et al.*, 2017).

Fermented food has its prestige and value as the process increases the shelf life and safety of the food, as well as its quality parameters, for instance, texture, taste, flavour, odour, and colour (Marco *et al.*, 2017). The fermentation process may also enhance the nutritional and health values of the food, and retain viable microorganisms and their metabolites, *e.g.*, LAB. *Belacan* is fermented shrimp

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paste commonly consumed in Malaysia, besides other fermented seafood products, such as *budu*, *cincalok*, and *pekasam*. During the production of *belacan*, small shrimps, such as *Acetes* spp. or mysids, will be washed and mixed with salt in a particular ratio before being sun-dried and pounded into a paste. The paste will be stored in a container to initiate the fermentation. The process is repeated until the desired texture is achieved (Huda, 2012). However, despite its common consumption across the country, there have been minimal reports investigating potential probiotics isolated from *belacan*. For example, Haitham *et al.* (2017) focused on LAB that grows in a strict anaerobic environment, with no reports on their haemolytic properties, resistance patterns towards antibiotics, and antagonism properties. Therefore, the present work aimed to isolate LAB strains from *belacan*, and evaluate their potential probiotic properties.

Materials and methods

Sample collection

Belacan sample was purchased from a market in Negeri Sembilan, and transported to the Food Microbiology Laboratory, Universiti Sains Islam Malaysia, Bandar Baru Nilai, Nilai, Malaysia, for isolation and characterisation studies. The samples were stored in air-tight containers, and kept in the refrigerator until further analyses.

Isolation of lactic acid bacteria (LAB)

The isolation of LAB was done using MRS agar (de Man, Rogosa, Sharpe; Oxoid, Thermo Fisher Scientific). Ten grams of the sample were added to 90 mL of MRS broth (Oxoid, Thermo Fisher Scientific) before a serial dilution up to 10^5 was prepared from the mixture. Approximately 100 µL of the homogenised mixture was spread on MRS agar plates containing 0.8% calcium carbonate ($CaCO_3$), and incubated at 37°C for 48 h. The white and creamy colonies with different morphologies and dissolved calcium circles were randomly chosen and subcultured on MRS agar plates until a pure colony was obtained. Commercial probiotics *Lactobacillus casei* strain Shirota (Yakult, Japan) was used as a reference strain.

All isolates were tested for their catalase activity using the slide method. A small amount of isolate was picked up using a sterile inoculating loop, and placed on the surface of a slide before the addition of a single drop of 3% hydrogen peroxide (H_2O_2) solution. The fluid was observed for the absence of bubble formation which indicated a negative catalase test.

Each pure isolate with a negative catalase test was examined for the Gram-staining reaction and cell morphology. Only catalase-negative and Gram-positive isolates were selected and stored in MRS broth in 20% (v/v) glycerol at -80°C. The isolates were revived before each experiment by subculturing twice on MRS agar, and inoculated in MRS broth.

Determination of acidic pH tolerance

The isolates were examined for their tolerance to acidic pH following Zhang *et al*. (2016) with slight modification. About 100 µL of the overnight culture of LAB equivalent to 0.5 McFarland standard or 10^8 colony forming units (CFU/mL) was transferred into 900 µL of MRS broth adjusted to pH 2.5, 3, and 6.2 (control) using hydrochloric acid (HCl), and incubated at 37°C for 3 h. The survivability of the isolates towards different pHs after 3 h was determined by serially diluting and inoculating the culture on MRS agar plates, and incubation for 48 h. Colonies formed were then counted and compared to the control. The experiment was performed in duplicates with three repetitions, and *L. casei* strain Shirota (Yakult, Japan) was used as a reference strain. The survival rate was reported as log values of CFU/mL. Isolates with survival rates above 90% were selected for further investigation.

Determination of bile tolerance

The tolerance of each isolate towards bile was studied following Kumar and Kumar (2015) with slight modifications. Initially, 25 µL of the overnight-grown bacterial suspensions equivalent to 0.5 McFarland standard were inoculated into 1 mL of MRS broth without bile salt (control) and with bile salt (Oxoid, Thermo Fisher Scientific) adjusted to 0.3%. All isolates were incubated at 37°C, and the absorbance was recorded at 600 nm after 7 and 24 h incubation. The absorbance was compared to a control culture with the absence of bile salt. The experiments were performed in three independent repetitions with duplicates for each experiment, and *L. casei* strain Shirota (Yakult, Japan) as the reference strain. Isolates with survival rates above 50% were selected for further investigation.

Determination of haemolytic activity

The selected isolates with promising acidic pH and bile tolerance were streaked on the surface of 5% sheep blood agar plates (Oxoid, Thermo Fisher Scientific) before incubation at 37°C for 48 h. The agar was observed for signs of β-haemolysis (clear zones around colonies), α-haemolysis (green-hued zones around colonies), or γ-haemolysis (no zones around colonies). The experiment was done in triplicates, and only isolates with α-haemolysis and γ-haemolytic properties were evaluated for the next characteristics.

Determination of antagonism properties

The antagonistic interaction of selected isolates against two Gram-positive bacteria (*Bacillus cereus* ATCC® 11778™ and *Staphylococcus aureus* NCTC 6571) and two Gram-negative bacteria (*Escherichia coli* ATCC® 25922™ and *Salmonella* Typhimurium) were determined by the spot overlay method following the method described by Shokryazdan *et al*. (2014) with slight modifications. Overnight cultures grown in MRS broth were adjusted to 0.5 McFarland standard, and spotted on MRS agar plates. The plates were air-dried for 30 min at room temperature prior to incubation at 37°C for 24 h. Colonies developed were overlaid with 10 mL of molten MH agar (Muller Hinton; Merck Millipore), seeded with 1% pathogenic bacteria (10^6 CFU/mL), and were further incubated for another 24 h at 37°C. Ampicillin disc (10 µg; Oxoid, Thermo Fisher Scientific) was used as the positive control. MRS broth was used as the negative control, while *L. casei* strain Shirota (Yakult, Japan) was the reference strain. The inhibition zones produced were measured from the edge of the isolate to the edge of the clear zone. The inhibition zones were scored as low, intermediate, and strong if the sizes were less than 10 mm, between 10 to 20 mm, and more than 20 mm (Shokryazdan *et al*., 2014).

Determination of antibiotic resistance patterns

The antibiotic resistance patterns of all strains were determined following the method described by Angmo *et al.* (2016) with slight modifications. The antibiotic discs (Oxoid, Thermo Fisher Scientific) used were ampicillin (10 µg), bacitracin (10 µg), chloramphenicol (10 µg), nalidixic acid (30 µg), penicillin G (10 units), streptomycin (10 µg), tetracycline (30 µg), and vancomycin (30 µg). MRS agar plates were spread with 100 µL of overnight-grown bacterial suspension equivalent to 0.5 McFarland standard using a sterile cotton swab. Then, different antibiotic discs containing ampicillin (10 µg), bacitracin (10 µg), chloramphenicol (10 µg), nalidixic acid (30 µg), penicillin G (10 units), streptomycin (10 µg), tetracycline (30 µg), and vancomycin (30 µg) were placed on the inoculated plates under sterile conditions. All plates were incubated at 37°C for 48 h before the diameter (mm) of the inhibition zone was measured.

Molecular identification using 16S rRNA gene

The genomic DNA of each isolate was extracted using the One-Tube Bacterial Genomic DNA Extraction Kit (Bio Basic, Canada) following the manufacturer's protocol with slight amendments. Purified DNA was used as a template to amplify a segment of the 16S rRNA gene by the PCR technique using the universal prokaryotic primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') (Lane, 1991). The PCR mixture consisted of 0.5 µL of the forward and reverse primers, 1 µL of template DNA, and 12.5 µL of MyTaq Red Mix (Bioline, UK). Autoclaved distilled water was added to the mixture to make up the volume to 25 µL. The PCR was operated under the following conditions: a cycle of initial denaturation at 95°C (1 min), 35 cycles of denaturation at 95°C (15 s), annealing at 50°C (15 s), and extension at 72°C (10 s). The size of the PCR products was approximately 1,500 bp, and they were commercially sequenced by Macrogen Laboratory, South Korea.

The amplified 16S rRNA gene sequences were further analysed using the nucleotide blast program provided by the online Basic Local Alignment Search Tool (BLAST) from the National Centre for Biotechnology Information (NCBI). Multiple sequence alignment of the isolates with closely related species, and the construction of a phylogenetic tree by the Maximum Likelihood method were performed using MEGA X (Kumar *et al*., 2018).

Statistical analysis

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS) version 24.0. Data were statistically analysed using one-way ANOVA and independent *t*-test. A significant difference was considered at the level $p < 0.05$.

Results and discussion

Isolation of LAB

A total of 17 pure isolates were successfully tested as exhibiting catalase-negative and Gram-positive characteristics, and labelled as BE1 to BE17. The colonies were morphologically round, white, or cream-coloured, with a raised or convex elevation, and rod-shaped.

Determination of acidic pH tolerance

The 17 BE isolates were further examined for their resistance after 3 h incubation in acidic environments, reflecting the gastric residence time (Maurer *et al*., 2015). Figure 1 shows the effect of different pH values on the survival of BE isolates and *L. casei* after 3 h incubation at 37°C. Only three isolates (BE3, BE7, and BE16) showed resistance values above 90% at pH 3 and 2.5. The results indicated that no significant difference ($p > 0.05$) was observed for BE3, BE16, and *L. casei* resistance when the pH decreased from 3.0 to 2.5. However, BE7 isolate survival decreased significantly ($p < 0.05$) when exposed to pH 2.5. Meanwhile, no significant effect ($p > 0.05$) was noted in different isolates on resistance percentage for the three isolates and *L. casei* in both treatment conditions.

The high survival rates of the three isolates compared to the remaining 14 isolates tested could have been due to the ability to adjust their internal pHs (Ng *et al*., 2015). The survival capability during gastric transit is one of the required criteria of a probiotic candidate (FAO/WHO, 2002). LAB must withstand a strongly acidic environment, which varies between pH 1.5 to 3.0. before they can function in the intestinal tract (Liong and Shah, 2005). In general, LAB can induce an acid tolerance response under acidic stress, resulting in pH homeostasis and a repair process that will eventually cause them to resist low pH (Aarti *et al*., 2017). The mechanisms include cell membrane structures that are highly impermeable and modulation of membrane channel size to restrict the flow of protons inside the cell; creating chemiosmotic gradients through potassium ATPases to bounce the inflow of protons, pumping out the excess proton by proton pump; and modulation of fatty acid structure to sustain the integrity and fluidity of cell membranes.

Generally, acid resistance capacities of isolates are strain- and species-dependent with general decrement below pH 3.0 (Corcoran *et al*., 2005; Nami *et al*., 2019). Our preliminary study of acid tolerance proved that the isolate could not withstand pH 2 and below. The findings were almost comparable to Yu *et al*. (2013) who reported that seven *L. plantarum* strains could tolerate the acidic conditions at pH 3, but their growths were strongly influenced at pH 2.0. Likewise, Sagdic *et al*. (2014) revealed that *L. plantarum* strains could grow at pH 2.5, but several *L. casei*, *L. brevis*, and *L. buchneri* strains failed to survive. Meanwhile, Haitham *et al*. (2017) discovered that 13 LAB isolates survived the acidic environment between pH 2.0 to 4.0.

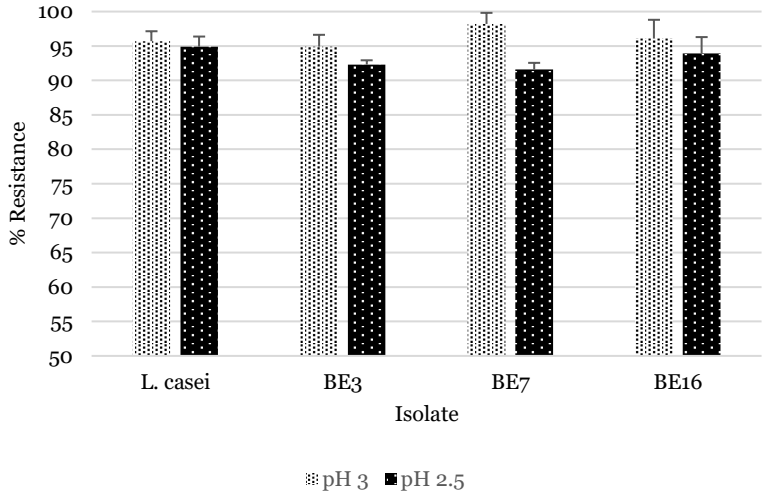

Figure 1. Effect of different pH values on survival of BE isolates and *L. casei* after incubation at 37°C.

Determination of bile tolerance

The isolates that survived acidic pH were further tested for their bile tolerance at 0.3% bile salt concentration. In the present work, the data were recorded at 7 h, reflecting the condition in the intestines (Fallingborg *et al*., 1990; Maurer *et al*.,

2015) and at 24 h. Figure 2 shows the effect of 0.3% bile salt on the survival of BE isolates and *L. casei* after 7 and 24 h incubation at 37°C. The results revealed that BE7 tolerance to 0.3% bile salt at 7 h was significantly lower ($p < 0.05$) than BE3 and *L. casei.* The resistance percentage of all isolates was still above 50% after 24 h exposure to 0.3% bile salt, and not significantly different from one another ($p > 0.05$). However, BE3 isolate survival decreased significantly ($p < 0.05$) when exposed to 0.3% bile salts for a longer duration (24 h).

This agreed with Gharbi *et al*. (2019) who revealed that their *Lactobacillus* strains exhibited tolerance to bile conditions with a survival rate between 53 and 108%. The lower resistance of *L. mesenteroides* (71.4%) and *Pediococcus pentosaceus* (51.89%) isolated from Tunisian bovine and turkey meat sausages was reported by Abid *et al*. (2018) at 0.3% bile salt concentration after incubation for 24 h at 37°C. One of the challenges to probiotic survival in the small intestine is bile salt. Besides gastric secretions, bile is produced as a bactericidal agent to fight potential pathogens that might cause foodborne illnesses (Merritt and Donaldson, 2009). Tolerance to bile salt becomes a requirement before probiotics can colonise and perform their metabolic activities (Shehata *et al*., 2016).

The resistance to bile salts, which commonly act as detergents that destroy the bacterial cell membrane, could be due to the presence of polysaccharides on the outer cell membrane of the probiotics (Tarique *et al*., 2022). Although the proposed bile salt concentrations to evaluate the probiotic characteristics are between 0.15 to 0.5%, isolates showing above 50% survival rates at 0.3% bile salt concentration are adequate to be considered bile resistant (Kumar and Kumar, 2015; Papadimitriou *et al*., 2015). The high percentage of survival indicates higher possible recovery of the isolates during the passage through the small intestine (Song *et al*., 2015).

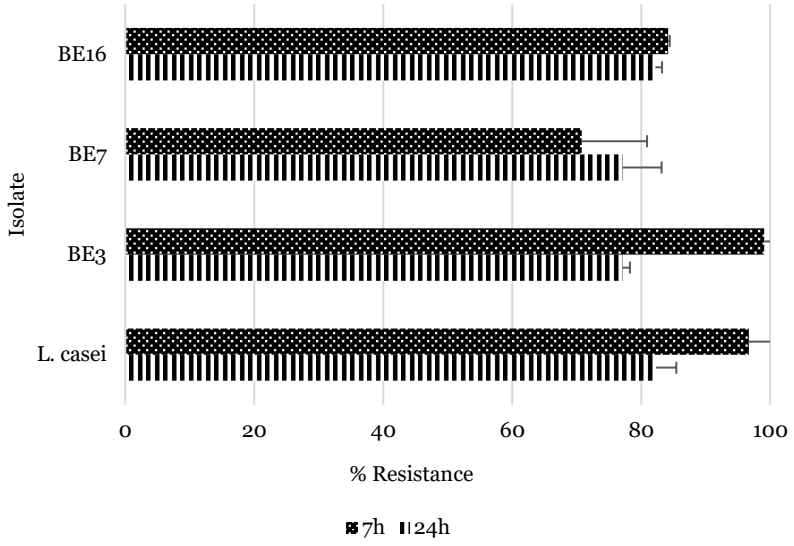

Figure 2. Effect of different incubation periods on survival of BE isolates and *L. casei* after incubation at 37°C.

Determination of haemolytic activity

The haemolytic properties of BE isolates were tested using 5% sheep blood agar plates. Haemolysis in blood agar is the destruction of red blood cell components, which could be due to the production of haemolytic proteins by the bacteria. FAO/WHO (2002) acclaimed the haemolysis test to confirm the safety of potential probiotics. Probiotics should not exhibit haemolytic ability to prevent any invasive disease due to the breakdown of the host's epithelial cells (Ida Muryany *et al*., 2017). The results of the present work indicated that all three isolates showed α-haemolysis. Some studies recommend that α-haemolytic bacterial strains are considered harmless for the development of probiotics as the isolates may harbour very minimal virulence ability (Argyri *et al*., 2013; Touret *et al*., 2018), and are common among lactobacilli from foods and dairy products (Halder *et al*., 2017). Nonetheless, the results differed from Abushelaibi *et al.* (2017) who reported that two of their nine LAB isolates were able to perform the β-haemolytic activity.

Determination of antagonism properties

Table 1 shows the antagonism properties of BE isolates against four pathogenic strains after incubation at 37°C for 24 h. All three isolates inhibited the growth of all pathogens to varying degrees, as indicated by the inhibition zones, which ranged from 8.0 to 14.2 mm, and were strain-dependent (Kumar and Kumar, 2015; Chopade *et al*., 2019). Although the isolates displayed low inhibition zones against *B. cereus* (inhibition zones of less than 10 mm), their activity was significantly higher than the positive control and reference strain ($p < 0.05$). The findings showed that BE3 caused significantly ($p < 0.05$) higher inhibition against *S. aureus, E. coli*, and *S.* Typhimurium (inhibition zones of more than 10 mm) compared to *B. cereus*. However, BE7 and BE16 showed significant inhibitory activities only against *E. coli* and *S.* Typhimurium in comparison against *B. cereus*. Meanwhile, BE3 produced the largest zone of inhibition (14.2 mm) against *E. coli.*

The antagonistic properties of LAB strains towards pathogenic bacteria may be due to nutrient constraints or the production of antimicrobial substances, such as hydrogen peroxide, bacteriocin-like molecules, and organic acids (Shokryazdan *et al*., 2014; Chopade *et al*., 2019). Organic acids produced by LAB decrease the pH, thus inhibiting the growth or killing the pathogens. Probiotic's ability to produce these substances is one of the essential attributes to compete with pathogens, and exert their advantageous effects beneficial to the host (Argyri *et al*., 2013). The substances need to penetrate the pathogen's outer membrane structures, comprising of highly impermeable hydrophobic lipid bilayer and proteins, which form pores that could allow molecules of very specific size to pass through (Delcour, 2009). A similar broad inhibitory spectrum of LAB has been reported by Shokryazdan *et al*. (2014) and Gao and Li (2018). However, this pattern was contradictory to that of Chopade *et al*. (2019) who reported that one or two of their isolates did not show any antagonistic activity to several or all pathogens tested, while the other inhibited Gram-negative bacteria better than Gram-positive bacteria.

Table 1. Mean inhibition zone (mm) of BE isolates and *L. casei* against pathogenic bacteria.

Isolate	*B. cereus*	*S. aureus*	*E. coli*	*S.* Typhimurium
BE3	8.8 ± 0.75^{a}	10.2 ± 0.98^{ab}	14.2 ± 0.75^{a}	12 ± 0.63^{a}
BE7	8.0 ± 0.63^{a}	10.6 ± 1.14^{ab}	11.5 ± 2.51^{ac}	13.2 ± 1.64^{ab}
BE16	8.3 ± 0.82^{a}	10.3 ± 1.97^{ab}	11.8 ± 2.48^{ad}	11.8 ± 1.64^{a}
L. casei	6.2 ± 0.75^{b}	8.2 ± 1.48^{a}	9.7 ± 2.34^{bcd}	10.2 ± 1.17^{ac}
Ampicillin (10 µg)	1.5 ± 0.55^{c}	11 ± 0.71^{b}	6.8 ± 0.75^{b}	11.5 ± 0.84^{a}

Values are mean of two independent experiments, each in triplicates ± SD. Means with different lowercase superscripts in the same column are significantly different ($p < 0.05$).

Determination of antibiotic resistance patterns

The isolates were tested for their antibiotic resistance patterns using the disc diffusion assay. Table 2 shows the diameter of the inhibitory zones (mm) among the isolates. Diameters of 20 mm and above are considered susceptible, between 15 to 19 mm as intermediate, and 14 mm and below as resistant (Sharma *et al*., 2016). Based on the findings, the resistant patterns of BE isolates were strain-dependent. Although all three LAB strains were susceptible to ampicillin and chloramphenicol, and resistant to nalidixic acid, streptomycin, and vancomycin, they showed different resistance patterns towards bacitracin, penicillin G, and tetracycline.

These results were comparable to those of Sharma *et al*. (2016) who discovered that lactobacilli were also resistant to similar types of antibiotics (nalidixic acid, streptomycin, and vancomycin) albeit displaying a lower resistant profile or sensitive towards ampicillin, chloramphenicol, penicillin, and tetracycline. Meanwhile, Aarti *et al*. (2017) reported that *L. brevis* strain LAP2 isolated from north-east Indian fermented fish known as *hentak* was sensitive to the antibiotics tested. The resistant ability of a bacterial strain to several antibiotics could be due to various reasons, either related to the antibiotics or the bacteria itself. The former involves the nature of the antibiotics and their ability to interfere with the production of bacterial cell walls, inhibition of

Table 2. Assessment of antibiotic resistance patterns of BE isolates and *L. casei* against eight different types of antibiotics.

	BE3	**BE7**	**BE16**	***L. casei***
Ampicillin	S	S	S	S
Bacitracin	I	R	I	S
Chloramphenicol	S	S	S	S
Nalidixic acid	R	R	R	R
Penicillin G	S	I	S	S
Streptomycin	R	R	R	R
Tetracycline	I	I	S	S
Vancomycin	R	R	R	R

S = susceptible (inhibition zone ≥ 20 mm); I = intermediate (inhibition zone 15 - 19 mm; and R = resistant (inhibition zone ≤ 14 mm) (Sharma *et al.*, 2016).

bacterial metabolic pathways, synthesis of bacterial proteins and nucleic acids, and modification of bacterial cell membranes. Meanwhile, the latter entails the structure of the cell wall, the permeability of the membrane, and the efflux mechanism (Shaikh *et al*., 2015; Abid *et al*., 2018).

To date, there are growing concerns about the ability of LAB isolated from food to act as a reservoir for antibiotic resistance genes and transfer to pathogenic bacteria, either during the manufacturing of food or during passage through the digestive tract (Ammor *et al*., 2007). The concern also includes the possibility of LAB transferring its resistant genes to microbial communities in the human intestine (Shazali *et al.*, 2014). However, the extensive use of LAB in fermented foods has a good long record of safety, and is designated as generally recognised as safe (GRAS), as most of the strains contain intrinsic resistance genes that are not transferable horizontally (Ammor *et al*., 2007; Jose *et al*., 2015).

Genotypic identification of LAB

The BLAST results revealed that all isolates were identified as *L. plantarum*, with 98 to 100% similarities to the GenBank nucleotide sequences database. All the BE3, BE7, and BE16 sequences were deposited into GenBank, with accession numbers MT163337, MT163338, and MT163339. The phylogenetic tree of 16S rRNA sequences of the three BE isolates and eight *Lactobacillus* strains obtained from the GenBank was constructed using MEGA X, and is displayed in Figure 3. *S. aureus* KC928093.1 was used as the outgroup.

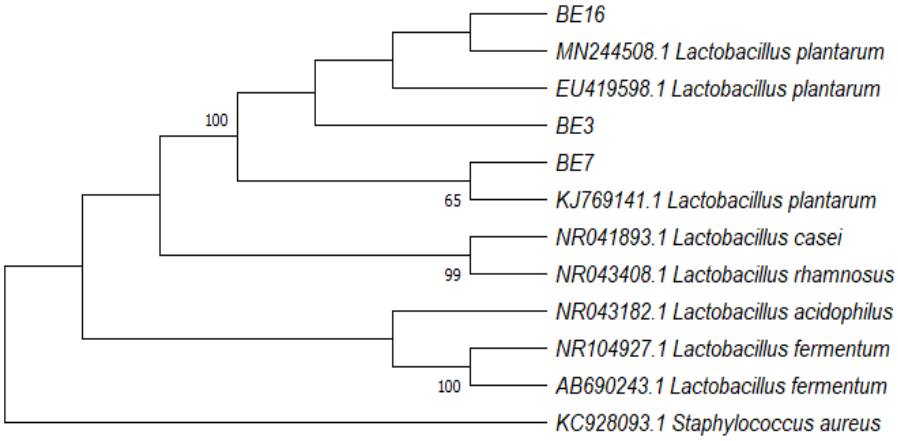

Figure 3. Maximum likelihood phylogenetic tree of BE isolates and reference strains based on the 16S rRNA gene sequence analysis.

Similarly, a study by Le and Yang (2018) concluded that one of the dominant isolates of their salted-fermented shrimps was *L. plantarum*, besides three other isolates that were close *to L. sakei*. In contrast, a study by Kobayashi *et al*. (2003) using 16S rRNA molecular identification discovered different species in their *terasi* shrimp of the genus *Tetragenococcus*, *i.e*., *T. muriaticus* and *T. halophilus.* Meanwhile, Daroonpunt *et al*. (2016) indicated that the microbial flora of Thai traditional fermented shrimp paste (*ka-pi*) varied and excluded the presence of LAB. Bacterial identification using the molecular method is vital to identify the genus and species of unknown isolates accurately (Shehata *et al*., 2016; Ida Muryany *et al*., 2017). Genotypic characterisation of bacteria based on the 16S rRNA gene sequence has been extensively used because the gene is present in nearly all bacteria, besides having a sluggish evolutionary rate, and a constant role and an adequate gene size (roughly 1,500 bp) sufficient for informatic purposes (Janda and Abbott, 2007).

Conclusion

Research on probiotic foods is performed worldwide due to the rising demand for products that not only nourish but also proffer health benefits. In the present work, three of 17 LAB strains isolated from Malaysian fermented shrimp paste (*belacan*) were successfully characterised for their probiotic potential. The isolates were identified as *L. plantarum* strain BE3 (MT163337), *L. plantarum* strain BE7 (MT163338), and *L. plantarum* strain BE16 (MT163339). All isolates exhibited promising probiotic characteristics, and are considered safe for consumption. More studies should be carried out to explore the interaction of these isolates with the intestinal cells, as well as their impact on the host's gene expressions and the modulation of cholesterol levels, in the quest to further understand the health benefits of these isolates.

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